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Metabolic parameters and dry matter intake of ewes treated with butaphosphan and cyanocobalamin in the early postpartum period

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ABSTRACT

The objective of this study was to determine the effects of butaphosphan and cyanocobalamin injection on the dry matter intake and metabolic parameters of early postpartum ewes. Eighteen pregnant ewes (Texel breed) were divided into two experimental groups, at calving day: (1) Treatment Group (BTC Group; $n = 9$), which received 3 injections of butaphosphan (1000 mg) and cyanocobalamin (0.5 mg) combination, every two days after calving (day 0–4 postpartum); and (2) Control Group (CTL Group; $n = 9$), which received injections of saline solution (NaCl 0.9%) in the same way that the BTC Group. Blood samples were collected once a day, from day 1 to day 7 postpartum to determine concentrations of glucose, non-esterified fatty acids (NEFA), acetone, beta-hydroxybutyrate (BHB), calcium (Ca) and phosphorus (P). The dry matter intake was higher ($P = 0.023$) in the BTC group (2.9 ± 0.2 *vs.* 2.0 ± 0.2 kg/day) while the NEFA concentration in this group was lower (0.47 ± 0.02 *vs.* 0.56 ± 0.02 mmol/L, $P = 0.006$). The acetone blood concentration decreased on the BTC group ($P = 0.068$) in the first week after calving (3.16 ± 0.34 mg/dL and 4.05 ± 0.33 mg/dL BTC and CTL group, respectively). The glucose and phosphorus concentration of the BTC group were higher ($P = 0.027$ and $P < 0.001$, respectively) than in the CTR group. The calcium and BHB concentrations decreased after calving in all ewes, but were not different between groups. In conclusion, butaphosphan and cyanocobalamin injections in the early postpartum period in ewes improved the energetic status and had an orexigenic effect.

1. Introduction

Physiological conditions of ketonemia are common in ruminants and include nutritional deficiency, bovine ketosis and ovine pregnancy toxemia (Bruss, 1997). These diseases are the result of the excessive lipid mobilization due to the negative energy balance (NEB) and of

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the increased blood levels of non-esterified fatty acids (NEFAs), which can be completely oxidized to CO_2 and H_2O by Krebs cycle (KC), or partially oxidized to ketone bodies (Caldeira, 2005; Zammit, 1990), as acetoacetate, beta-hydroxybutyrate (BHB) and acetone (Church, 1993; Drackley et al., 2001). The acetoacetate is unstable and can be converted to acetone and carbon dioxide (Fukao et al., 2004). The acetone by addition of H^+, is transformed into the intermediate isopropanol (Adler et al., 1955), responsible for signals encephalopathy in cases of high levels of ketone bodies during severe NEB (Pethick et al., 1983). Therefore, the ketonemia is associated with anorectic effects and decreased dry matter intake (DMI) (Scharrer, 1999).

Strategies as the control of body condition (Sarginson, 2007) and the use of bovine somatotropin (Lucci et al., 1998) have been used to minimize the NEB in ruminants. Another alternative is the combination of butaphosphan and cyanocobalamin used mainly to improve the immune and energetic status in cows (Flasshoff, 1974; Larscheid, 1994; Simon, 1990). However, the exact mode of action of these substances in combination is not yet fully known. What is known is that cyanocobalamin is a cofactor of the enzyme methylmalonyl-CoA mutase, which is essential for entry of propionate into the KC and gluconeogenesis (Mcdowell, 2000). As a limited amount of glucose is absorbed by intestine, soon after calving, when there is a greater energy requirement, the animal becomes dependent of the glucose from gluconeogenesis (Reynolds, 2006). Furthermore, an insufficient supply of cyanocobalamin, especially after calving, might decrease the function of methylmalonyl-CoA mutase and the cell energy synthesis (Kennedy et al., 1990). The decrease of the KC activity causes an accumulation of acetyl-CoA from the hepatic β-oxidation of NEFA, intensifying ketogenesis, and reducing the DMI (Baird, 1982). Butaphosphan, an organic source of phosphorus, is important in cellular energy metabolism and serves as substrate for the ADP/ATP cycle, synthesis of creatine phosphate and nucleotides involved in the hormonal action (cAMP, cGMP) (Cunningham, 2002). Therefore, it can serve as a regulator of gluconeogenesis and glycolysis rates that depend on the availability of phosphorus (Berg et al., 2006). Although cyanocobalamin and butaphosphan can directly interfere with certain metabolic pathways, it is assumed that there is still an indirect effect on hepatic metabolism, mainly in the processes of β-oxidation and ketogenesis, which could explain the improvement in the energetic condition of the animals treated with these substances (Furll et al., 2010; Rollin et al., 2010). In addition, these substances can reduce the expression of ACSL1 mRNA (acyl-CoA synthetase), an enzyme involved in β-oxidation (Kreipe et al., 2011).

One of the main application fields of this metaphylactic strategy is the treatment of ketosis in dairy cows (Rollin et al., 2010), besides improving the energy synthesis during the peripartum (Furll et al., 2010). Furthermore, other benefits to ruminants include the prevention of milk fever (Schuh, 1994), the improvement of the productive and reproductive performance (Flasshoff, 1974), the decreasing of stress (Simon, 1990), and a faster recovery after surgical procedures (Furll et al., 2006, 2010). In sheep, its use has been restricted to animals submitted to estrus synchronization protocols in the search for a better reproductive performance (Lopes et al., 2010).

The aim of this study was to evaluate the effects of a new metaphylactic strategy with butaphosphan and cyanocobalamin on serum levels of NEFA, acetone, BHB and the DMI in postpartum ewes. Our hypothesis is that the synergistic action of these substances improves the gluconeogenesis, reduces the severity of NEB, plasma concentrations of acetone and BHB, increasing the DMI.

2. Material and methods

2.1. Animal welfare

All experimental procedures were approved by the Ethics Committee on Animal Experimentation and are registered at Coordinating Council for Education, Research and Extension of the Federal University of Pelotas, Rio Grande do Sul, Brazil.

2.2. Animals and treatments

This study was conducted in a farm located in southern Brazil (31°56′ S, 52°14′ W), with mean annual temperature of 16.3 °C and average annual precipitation of 1.379 mm, characteristics of a humid subtropical climate. The experiment was realized from August to October 2010, under a thermal range from 10.7 to 21.8 °C, with eighteen pregnant ewes of the Texel breed, from second or third calving of a single lamb. The ewes were kept under the same management conditions, during pre and post-partum (confined in individual stalls). The animals were randomly divided into two groups, immediately after calving: (1) Treatment Group (BTC group, $n = 9$, BW mean of 54.68 ± 1.76 kg), that received 1000 mg of butaphosphan and 0.5 mg of cyanocobalamin (im, Catosal B_{12}®, Bayer Health Care, Sao Paulo, Brazil), (2) Control Group (CTL group, $n = 9$, BW mean of 52.19 ± 1.84 kg), that received the same amount of physiological solution (NaCl 0.9%). Three doses were administrated every 2 days after calving.

2.3. Dry matter intake

The animals were subjected to a confinement system, in individual stalls, from calving to 7 days postpartum. During this period they were fed *ad libitum*, twice a day (at 7 a.m. and 7 p.m.) with a total mix, consisting of forage (corn silage) and concentrate (Table 1). The total mix was comprised of 60% of dry matter and 40% of concentrate. The total mix was supplied 60% at morning (7 a.m.) and 40% at night (7 p.m.). The estimated DMI was based on 3% of the BW. Before each daily supply, the leftover was weighed, and daily DMI estimated. The amount offered was adjusted daily, so that the leftovers remained between 10% and 20% of the total supply.

2.4. Collection of blood samples

Blood samples were daily collected, from first to seventh postpartum day, and placed into three tubes previously identified: tube 1, containing KF (potassium fluoride) glycolytic pathway inhibitor, for analysis of glucose; tube 2, containing sodium fluoride, for analysis of acetone; and tube 3, without anticoagulant for the analysis of NEFA, BHB, Ca and P. The second tube was immediately frozen at −20 °C. The samples from tubes 1 and 3 were centrifuged at 3500 rpm for 15 min immediately after collection. The plasma and serum were distributed at duplicate in previously identified tubes and frozen at −20 °C.

2.5. Biochemical analysis

The concentrations of glucose, calcium (Ca) and phosphorus (P) were analyzed by enzymatic colorimetric assay quantified by spectrophotometer (FEMTO 700 Plus, Femto Ind. e Com. de Instrumentos Ltda., São Paulo, Brazil). The reagents were handled according to the manufacturer's instructions (Labtest®, Lagoa Santa, Brasil). Non-esterified fatty acid and BHB were analyzed by a commercial kit (Wako NEFA-HR, Wako Chemicals

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Table 1
Composition and assurance levels of the concentrate used in the feed of the ewes (Ration Commercial Irgovino®).

Components		Assurance levels	
		Minimum	Maximum
Ground soybean hulls	Unit	–	120.00 g/kg
Ground corn	Crude protein	150.00 g/kg	–
Defatted rice bran	Mineral matter	–	160.00 g/kg
Soybean meal	Calcium	15.00 g/kg	19.00 g/kg
Wheat meal	Phosphorous	7.00 g/kg	–
Mineral salt	Fiber matter	–	140.00 g/kg
Calcium carbonate	ADF	–	170.00 g/kg
Vitamin–mineral premix	Ether extract	25 g/kg	–
ADE vitamin	Sulphur	2.50 g/kg	–
	Vitamin A	3750.00U.i./kg	–
	Vitamin D3	1250.00U.i./kg	–
	Vitamin E	10.00U.i./kg	–
	Iodine	1.00 mg/kg	–
	Selenium	0.25 mg/kg	–
	Zinc	37.00 mg/kg	–
	Cobalt	0.50 mg/kg	–
	Iron	50.00 mg/kg	–
	Magnesium	2.50 g/kg	–

USA®, Richmond, EUA and Randox®, Randox Laboratories E.U.A.®, Ocean-side, CA, USA, respectively), according to (Ballou et al., 2009). Acetone was analyzed by gas chromatography (Sato and Shiogama, 2009). Coefficients of variation were lower 10% for all assays.

2.6. Statistical analysis

Data involving repeated measures over time (*e.g.*, glucose, Ca, P, NEFA, BHB, acetone and DMI), considering the daily average of the animals in each group, were analyzed by MIXED procedure of SAS® version 9.0 (SAS Institute Inc., Cary, NC, USA), to evaluate the main effects of treatment, time (days), and their interactions (Littell et al., 1998). When interactions were significant ($P < 0.05$) a least square difference test was used to detect treatment effects at each time.

3. Results

The dry matter intake of the BTC group (2.9 ± 0.2 kg/day) was higher ($P < 0.023$) than CTL group (2.0 ± 0.2 kg/day, Table 2) during first two days after calving ($P = 0.002$), according to Table 3. The BTC group had lower acetone concentration ($P = 0.068$) than CTR group (3.16 ± 0.34 mg/dL and 4.05 ± 0.33 mg/dL, respectively) during first week after calving (Table 2). This reduction was evident from day 5 after calving, when acetone concentration was lower ($P = 0.012$) to BTC group, than CTR group (Table 3). The glucose concentration to BTC group was higher (61.26 ± 1.94 mg/dL; $P = 0.027$), than to CTR group (55.10 ± 1.93 mg/dL). The average of the phosphorus concentration was higher ($P < 0.001$) to BTC group (7.40 ± 0.15 mg/dL), than to CTR group (6.60 ± 0.16 mg/dL; Table 2).

During the first week after calving the average of NEFA concentration to BTC group (0.47 ± 0.02 mmol/L) was lower ($P = 0.006$), than to CTR group (0.56 ± 0.02 mmol/L). The body weight of the ewes from BTC group (54.68 ± 1.76 kg; $P = 0.025$) was higher, than CTR group (52.19 ± 1.84 kg). The calcium and BHB concentrations decreased progressively

Table 2
Weekly average of metabolic parameters and dry matter intake (DMI) of ewes of the Texel breed that received injections of placebo (CTL group, $n = 09$) or 1000 mg of butaphosphan and 0.05 mg of cyanocobalamin (BTC group, $n = 09$) during first week postpartum.

Parameters[a]	Treatment[b]		Repeated measures[c]		
	CTL group	BTC group	Treatment	Period	Treat.*period
Ewes (n)	09	09			
Glucose (mg/dL)	55.10 ± 1.93	61.26 ± 1.94	0.027	0.144	0.738
NEFA (mmol/L)	0.56 ± 0.02	0.47 ± 0.02	0.006	0.229	0.830
BHB (mmol/L)	0.41 ± 0.03	0.42 ± 0.03	0.751	<0.001	0.860
Acetone (mg/dL)	4.05 ± 0.33	3.16 ± 0.34	0.068	0.760	0.020
Ca (mg/dL)	9.71 ± 0.40	9.78 ± 0.39	0.901	0.023	0.378
P (mg/dL)	6.60 ± 0.16	7.4 ± 0.15	<0.001	<0.001	0.207
DMI (kg)	2.0 ± 0.20	2.90 ± 0.20	0.023	0.001	0.002
BW (kg)	52.19 ± 1.84	54.68 ± 1.76	0.025	0.993	0.994

[a] Plasma concentration of glucose, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), acetone, calcium (Ca), phosphorus (P), dry matter intake (DMI) and body weight (BW) of the ewes during first week postpartum.
[b] Treatments consisted of three injections administered at days 0, 3 and 6 postpartum. CTL Group = saline solution ($n = 09$); BTC Group = 1000 mg of butaphosphan and 0.05 mg of cyanocobalamin ($n = 09$).
[c] Analysis of repeated measures by mixed model procedure, evaluating the main effects of treatment, time (days), and their interactions. Interactions were considered significant when $P < 0.05$.

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Table 3
Daily average of metabolic parameters and dry matter intake of Texel breed ewes, that received injections of placebo (CTL group, $n = 09$) or 1000 mg of butaphosphan and 0.05 mg of cyanocobalamin (BTC group, $n = 09$) during first week postpartum.

Parameters[a]	Treatment[b]	Period (days)							Repeated measures[c]
		1	2	3	4	5	6	7	Treatment*period
Glucose (mg/dL)	CTL Group	58.49 ± 5.03	60.64 ± 4.74	51.51 ± 4.74	55.72 ± 5.03	53.33 ± 5.02	54.26 ± 5.38	51.79 ± 5.81	0.738
	BTC Group	73.97 ± 5.38	59.41 ± 5.03	54.10 ± 5.38	66.70 ± 5.38	59.13 ± 5.38	60.08 ± 4.74	55.46 ± 4.74	
NEFA (mmol/L)	CTL Group	0.67 ± 0.06	0.61 ± 0.06	0.54 ± 0.06	0.50 ± 0.06	0.53 ± 0.07	0.59 ± 0.06	0.51 ± 0.06	0.830
	BTC Group	0.55 ± 0.06	0.50 ± 0.06	0.51 ± 0.06	0.50 ± 0.06	0.43 ± 0.06	0.41 ± 0.06	0.40 ± 0.07	
BHB (mmol/L)	CTL Group	0.63 ± 0.09	0.53 ± 0.11	0.16 ± 0.10	0.33 ± 0.09	0.47 ± 0.09	0.35 ± 0.10	0.41 ± 0.12	0.860
	BTC Group	0.69 ± 0.09	0.61 ± 0.10	0.28 ± 0.10	0.21 ± 0.09	0.41 ± 0.10	0.41 ± 0.09	0.40 ± 0.09	
Acetone (mg/dL)	CTL Group	2.40 ± 0.87	3.35 ± 0.87	4.29 ± 0.87	2.81 ± 0.87	5.26 ± 0.92[a]	5.57 ± 0.92[a]	4.69 ± 0.98[a]	0.020
	BTC Group	4.96 ± 0.98	2.66 ± 0.87	2.86 ± 0.92	4.02 ± 0.87	1.97 ± 0.92[b]	3.22 ± 0.92[b]	2.48 ± 0.87[b]	
Ca (mg/dL)	CTL Group	10.07 ± 0.84	9.87 ± 0.89	10.99 ± 0.96	10.56 ± 0.84	9.04 ± 0.84	8.47 ± 0.84	8.99 ± 0.84	0.378
	BTC Group	11.79 ± 0.79	9.45 ± 0.79	10.64 ± 1.05	8.92 ± 0.79	9.47 ± 0.84	8.00 ± 0.79	10.22 ± 0.79	
P (mg/dL)	CTL Group	8.20 ± 0.44	6.24 ± 0.42	5.10 ± 0.42	6.36 ± 0.42	5.60 ± 0.44	7.19 ± 0.44	7.55 ± 0.47	0.207
	BTC Group	9.60 ± 0.42	6.83 ± 0.42	6.19 ± 0.42	6.83 ± 0.42	7.60 ± 0.44	7.06 ± 0.42	7.73 ± 0.42	
DMI (kg)	CTL Group	1.11 ± 0.20[a]	1.03 ± 0.20[a]	2.50 ± 0.23	2.42 ± 0.20	2.48 ± 0.20	2.42 ± 0.21	2,69 ± 0.20	0.002
	BTC Group	2.87 ± 0.20[b]	2.87 ± 0.20[b]	2.95 ± 0.23	2.85 ± 0.20	2.83 ± 0.21	2.61 ± 0.21	2.95 ± 0.20	
BW (kg)	CTL Group	52.50 ± 2.20	53.43 ± 2.35	51.38 ± 2.20	51.38 ± 2.20	52.08 ± 2.33	52.62 ± 2.20	51.96 ± 2.33	0.994
	BTC Group	53.57 ± 2.35	54.89 ± 2.07	54.56 ± 2.07	55.00 ± 2.07	54.63 ± 2.20	55.13 ± 2.20	55.00 ± 2.20	

Different superscripts indicate differences at $P < 0.05$ between treatments in the specific period.

[a] Plasma concentration of glucose, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), acetone, calcium (Ca), phosphorus (P), dry matter intake (DMI) and body weight (BW) of ewes during first week postpartum.

[b] Treatments consisted of three injections administered at days 0, 3 and 6 postpartum. CTL Group = saline solution ($n = 09$); BTC Group = 1000 mg of butaphosphan and 0.05 mg of cyanocobalamin ($n = 09$).

[c] Analysis of repeated measures by mixed model procedure, evaluating the main effects of the interactions between treatment and period (days). Interactions were considered significant when $P < 0.05$.

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at days 1, 2 and 3 postpartum in all animals, but were not different between groups (Table 3).

4. Discussion

In this study, the increase of the DMI and decreased acetone levels during first week after calving in the ewes treated with butaphosphan and cyanocobalamin, suggest an improved energy status, mainly by increased of the DMI, which was associated with reduced lipid mobilization and ketogenesis. These results are similar to previous observations in dairy cows (Pereira et al., 2013; Rollin et al., 2010).

Besides the effect indirect of butaphosphan and cyanocobalamin into minimize NEB, we must consider that with the reduced levels of acetone, by the degradation of its molecule, fragments of three (C3) and two (C2) carbons are produced and may be incorporated in metabolic pathways (Kalapos, 1999). When acetone blood concentration exceeds 4 mmol/L, intended to form acetate, however, if is below this level, it becomes part of the route of transformation of ADP to ATP in the KC (Kosugi et al., 1986). In addition, the acetone is converted to methylglyoxal by products of cytochrome P450IIE1 gene in the liver (Casazza et al., 1984). Two-way of C3 fragments have been described, containing the common formation of pyruvate as a final product (Kalapos, 1999), which would have a fundamental role in the gluconeogenesis, as described in rodents and humans (Casazza et al., 1984; Kalapos et al., 1994).

Although acetone serves as a gluconeogenic substrate, it also can interact with other substances that have an effect on glucose metabolism during hunger (Kalapos et al., 1994). Thus, we believe in the orexigenic effect of the butaphosphan, increasing the DMI mainly during first 2 days after calving. Besides, our results indicate glucose levels increased ($P = 0.027$) in the treated animals (Table 2), being consistent with the mechanism of action of cyanocobalamin, which acts as a cofactor for methylmalonyl-CoA mutase (an enzyme that catalyzes the conversion of propionate to succinyl-CoA). This catalyzation is key for the entry of fat acids in the KC to be used in gluconeogenesis (Kennedy et al., 1990). This action favors the synthesis of energy, which can be increased after the 5th day postpartum, when the plasma acetone is less than 4 mmol/L, integrating the route of transformation from ADP to ATP in the KC (Kosugi et al., 1986). Cyanocobalamin is produced in the ruminant digestive system, but soon after calving its blood levels are low (Girard and Matte, 1999). This occurs because the prepartum is the period of greatest fetal growth and the partition of nutrients increases for the formation of colostrum (Duehlmeier et al., 2011; Schlumbohm and Harmeyer, 2003). Thus, the functions of cyanocobalamin are compromised and its supplementation becomes a potential alternative, which can, by the gluconeogenic action, supply the energy demanded from organism (Brozos et al., 2011).

Depending on the available energy, the body may choose the gluconeogenesis. But, the lipolysis mechanism can simultaneously be used to provide energy to peripheral tissues (Kalapos et al., 1996). In addition, the hepatocytes of animals with energy deficiency oxidize large quantities of fatty acids, increasing the rate of NADH/NAD in the mitochondria (Enjalbert et al., 2001). However, as ruminants, the hepatic conversion of acetoacetate to BHB and then acetone requires only NAD and it does not depend on the rate NADH/NAD mitochondrial, because its conversion is cytosolic (Koundakjian and Snoswell, 1970), the formation of the ketone bodies typically occurs under presence of glucogenic precursors.

The acetone blood concentration was less in the BTC group, from 4 days postpartum (Table 3), while on the days before, the level was increased (less than 4 mol/L) what may have contributed, together with butaphosphan and cyanocobalamin, for increase the glycemia after day 4 (Kalapos et al., 1996). Furthermore, we must consider that, gluconeogenic substrates may provide NADPH and H^+ to cytochrome P450s, which acts in the metabolism of acetone and reduces its blood levels, (Kalapos et al., 1994), corroborating with our results mainly from day 4 postpartum.

In other studies (Furll et al., 2010; Krdzalic and Curcic, 1976; Sarasola et al., 2008) realized with cows, the significant effects of combined butaphosphan and cyanocobalamin on blood concentrations of phosphorus, glucose and BHB were observed after treatment, but the extent of the effect was even greater some days after. This shows that some results are immediate and occur even during the metabolism of substances, during its half-life, but other physiological effects are only observed at medium term. This maybe is related to lower severity of NEB of the animals from this study, which gives them a faster recovery, compared to the energy deficit, impacting on variables such as milk production, not evaluated in this study in sheep, but proven in cows (Furll et al., 2010; Kreipe et al., 2011; Rollin et al., 2010).

According to Table 2, the phosphorus blood concentration of the BTC group was higher (7.40 ± 0.15 mg/dL, $P < 0.001$), than the CTL group (6.60 ± 0.16 mg/dL, $P < 0.001$). While Furll et al. (2010) found a similar result during a study in dairy cows, Delport et al. (2006) and Rollin et al. (2010) did not observed the same effect, because according to them, the injection of butaphosphan not cause an immediate increase of phosphorus in blood due to their organic and stable form.

Therefore, we can say that the metaphylactic strategy with butaphosphan and cyanocobalamin during the recent postpartum period of ewes, at dose used in this study (2 mL/10 kg PV), plays a key role for decrease of NEB to the reducing of the levels of ketone bodies and increase DMI, corroborating with several previous studies realized with dairy cows.

5. Conclusion

In conclusion, the treatment of ewes with the metaphylactic combination of butaphosphan and cyanocobalamin during first week postpartum, decreases serum concentrations of NEFA and acetone, and increases blood concentrations of P, glucose and dry matter intake, indicating an overall improved energy status.

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